REMARKS/ARGUMENTS

Status of the claims

Claims 30-40 are pending in the present application. Applicants acknowledge with appreciation the time taken by the Examiner to discuss the outstanding rejections in the telephone interview on December 17, 2008. The rejections under 35 U.S.C. § 112, first and second paragraphs, were discussed as detailed below.

The claims have been amended to clarify certain claim terms as detailed in the discussion below. In addition, claim 30 has been amended to clarify that the recombinant DNA vectors of the invention comprise a promoter operably linked to a heterologous *NHX1* polynucleotide sequence. Support for this amendment is found in claim 35 and in paragraph [22], which states that a heterologous coding sequence is one that is from a species different from the promoter or has been genetically engineered. Claim 35 has been amended to recite that the promoter is a plant promoter. Support for this amendment may be found, for example, in paragraphs [50] and [51]. No new matter has been added.

Rejections under 35 USC § 112, second paragraph

Claims 30 and 36 stand rejected because the term "identical" is allegedly ambiguous as to whether the term refers to structural or functional identity. The claims have been amended to clarify that the term refers to sequence (*i.e.*, structural) identity.

The claims were also rejected for reference to polypeptides up to 530 amino acids in length, when SEQ ID NO: 10 is 454 amino acids in length. To expedite prosecution, the claims are amended to refer to SEQ ID NO: 2, the full length NHX1 polypeptide, which is 538 amino acids in length.

Finally, the claims were rejected for use of open language, which allegedly rendered the claims unclear in view of the fact that the encoded polypeptides are less than 530 amino acids in length. Claim 36 now recites that the claimed recombinant vector comprises a *NHX1* polynucleotide that ***consists*** of a coding sequence encoding a C-terminally truncated NHX1 polypeptide that is, among other things, less than 530 amino acids in length. Thus, although the recombinant vector may comprise other components (*e.g.*, selectable markers, and

the like), the *NHX1* polynucleotides consist of a coding sequence that does not encode a full length polypeptide. Withdrawal of the rejection is respectfully requested.

Rejections under 35 USC § 112, first paragraph – new matter

The claims stand rejected for allegedly introducing new matter. Although applicants believe the claims previously presented are well supported by the specification, the claims have been amended to overcome these rejections.

The claims stand rejected because terms referring to "90% identical to SEQ ID NO: 10" in combination with various polypeptide lengths are allegedly not supported by the specification. To expedite prosecution, the claims now refer to SEQ ID NO: 2, which is the language from the un-amended claims.

The claims also stand rejected because the term "compared to a plant that lacks the *NHX1* polynucleotide sequence" is allegedly not supported. The Examiner acknowledges that the term "compared to a plant where the *NHX1* polynucleotide was not introduced" does find support in claim 1, as filed. The claims have been amended to use the latter term, except that "where" has been replaced with "into which," for the sake of clarity.

Rejection under 35 USC § 112, first paragraph – enablement

The Examiner has rejected claims 30, 33-36, 39, and 40 for allegedly lacking enablement. According to the Examiner, the specification does not disclose working examples of a NHX1 polypeptide with 90% identity to SEQ ID NO:10 or C terminal deletions to SEQ ID NO:10. The Examiner then points to data in the specification to assert that most site mutations do not increase Na+ tolerance, and that two of the N-terminal deletions reduce Na+ tolerance. The Examiner contends these data indicate that it is unpredictable which mutations and deletions to SEQ ID NO:10 will have increased Na+ tolerance. Applicants respectfully traverse the rejection.

The familiar *Wands* test for enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation (MPEP § 2164.01). As explained in MPEP § 2164.02, the specification need not contain a working example to meet this requirement.

As explained previously, the present specification teaches that C-terminally truncated NHX1 polypeptides, with up to at least 84 C-terminal amino acids deleted (*e.g.*, SEQ ID NO:10), lead to altered ion specificity and thereby confer better Na+ tolerance than full length NHX1. The Examiner appears to acknowledge this fact, but asserts that C-terminal deletions of SEQ ID NO: 10, do not show enhanced Na + tolerance. The Examiner's concern about the effect of deletions in SEQ ID NO: 10 is misplaced. It is clear that claim 1 is directed to vectors that encode C-terminal truncations of full length NHX1 polypeptides (*e.g.*, SEQ ID NO: 2). The claim does not recite a C-terminally deleted SEQ NO:10, which is an exemplary C-terminally deleted NHX1 polypeptide.

As noted above, the Examiner refers to data in Table 1 that show that a number of site mutations in NHX1 do not affect Na+ tolerance, as compared to the wild-type protein. This is used as evidence that the effect of mutations is allegedly unpredictable. As an initial matter, the invention as presently claimed is based on the discovery that ***C-terminally truncated proteins*** have altered ion specificity and increased Na+ tolerance. Thus, whether point mutations, alone, lead to increased Na+ tolerance is not relevant to the present claims.

More importantly, Table 1, far from supporting the Examiner's position, actually demonstrates why the rejection is improper. As noted previously, Table 1 provides the results of experiments in which yeast lacking the endogenous nhx1 gene were transformed with various mutant forms of *Arabidopsis* NHX1 and tested for salt tolerance. Table 1 indicates that nearly all of the 23 site mutations tested had no effect on Na+ tolerance, as compared to wild type NHX1. The results thus suggest that the function of NHX1 proteins is not generally affected by such changes. These data also provide important guidance to one of skill when introducing changes in the truncated polypeptides of the invention. For example, the results suggest that modifications to residues 85, 86, and 142 should be avoided since site mutations at these residues resulted in lower tolerance (*see*, SM-1 and SM-3 in Table 1).

Each of the 23 site mutations and 6 truncations described in Table 1 is precisely indicated in the NHX1 polypeptide structure disclosed in Figure 6. Figure 6 also localizes some of the transmembrane and functional domains of NHX1. The present disclosure thus teaches the effect of mutations in the NHX1 structure and links them to various functional domains in the

protein. Given the general knowledge and the present disclosure, one of skill could reasonably predict how certain modifications, and combinations thereof, would effect NHX1 structure and function. For example, one of skill in the art would recognize that hydrophobicity of amino acids is preferable in transmembrane regions, and therefore would not introduce a hydrophilic residue in such regions. Based on the present disclosure, one of skill would understand which regions of NHX1 are amendable to modification, and be able to design a functional variant C-terminally truncated NHX1 with the recited sequence identity.

Further, the specification describes straightforward methods to screen variants of SEQ ID NOs: 2 and 10 for Na+ tolerance. These methods include transforming yeast with the recombinant variant sequences and testing growth under different salt conditions (as in Example 7). Such assays are routine and fast, and thus would not constitute undue experimentation.

Finally, the present disclosure supplements the general familiarity of those of skill in the art with Na+/ H+ antiport proteins. As noted in paragraph [09] such genes had been cloned and characterized prior to the present invention.

Thus, the specification enables one of skill to practice the invention without undue experimentation. The disclosure indicates which residues are amenable to mutation without affecting Na+ tolerance, which provides important guidance to preparing variants of SEQ ID NO:10 and other C-terminally truncated NHX1 polypeptides. The specification also provides important information about the structure and function of various domains within the protein. Finally, to confirm predicted activity, one of skill could easily test whether a particular variant C-terminally deleted NHX1 polypeptide confers the desired phenotype.

In view of the foregoing comments, Applicants respectfully request withdrawal of the rejection under the first paragraph of 35 USC § 112 for enablement.

Rejection under 35 USC § 112, first paragraph – written description

The Examiner has rejected claims 30, 33-36, 39, and 40 as allegedly lacking written description. In response to previous arguments, the Examiner states that not a single species encompassed by the claims has been reduced to practice. The Examiner's position appears to be based on an interpretation that the claims are directed to truncations to SEQ ID

NO: 10. As noted above and clarified in the present claims, SEQ ID NO: 10 is an exemplary truncated polypeptide of the invention.

The test of written description is whether one of skill in the art would reasonably conclude that the inventor had possession of the claimed invention. As explained in MPEP § 2163, possession can be shown in a variety of ways, including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. Reduction to practice is ***not*** an absolute requirement. The standard is one of reasonableness, based on the understanding of one of skill in the art at the time of the invention.

As noted above, the specification describes the functional effect of 23 different site mutations and 6 truncations of NHX1. In particular, the specification teaches (i) that C-terminally deleted NHX1, including SEQ ID NO:10, confers higher Na+ tolerance than the full length NHX1, and (ii) that a number of residues can be modified without changing the Na+ tolerance function of NHX1. Moreover, the proposed structure of NHX1 is provided in Figure 6, which also locates the myriad mutations and truncations described in Table 1 relative to structural features of the NHX1 polypeptide. One of skill would reasonably view Figure 6 *as a whole*, in combination with the results disclosed in Table 1, and conclude that the inventors knew where modifications could be made to the NHX1 polypeptide in order to affect its Na+ tolerance activity. This is especially true given the general knowledge in the art regarding Na+/ H+ antiporter proteins, described in the specification.

The specification therefore describes identifying characteristics of functional NHX1 variants sufficient for one of skill to conclude that applicants were in possession of the claimed invention. In view of the foregoing comments, Applicants respectfully request withdrawal of the rejection under the first paragraph of 35 U.S.C. §112 for written description.

Rejection under 35 USC § 102

The pending claims stand rejected for allegedly being anticipated by Gaxiola *et al. Proc Nat Acad Sci* 96:1480-1485 (1999). Gaxiola *et al.* is cited to teaching the full length sequence of the AtNHX1 protein. As explained above, the present claims are directed to C-terminally truncated NHX1 polypeptides that are less than 530 amino acids in length and have altered ion selectivity. In light of the above, applicants believe the rejection is improper as applied to the pending claims.

Supplemental IDS

Enclosed with this response is a Supplemental IDS enclosing a copy of a Communication from the European Patent Office, citing two EMBL Genbank database accessions. Applicants respectfully submit that neither of these database accessions anticipate or render obvious the pending claims. In particular, EMBL Genbank accession no. O04655 (reference D4) is a predicted amino acid sequence of an open reading frame identified by computer algorithm in a large genomic insert in a BAC vector, generated as part of a project to sequence the *Arabidopsis* genome. The entire genomic sequence was submitted under accession AF007271, which is referred to in accession no. O04655.

Accession AF007271, as it appeared in June, 1997 is provided for the Examiner's convenience. There, it can be seen that the genomic insert is over 90,000 base pairs in length and comprises 16 predicted open reading frames. Thus, this prior art reference does not teach an isolated nucleic acid encoding the truncated NHX1 polypeptides of the invention, nor does it motivate one of skill in the art to prepare the DNA vectors of the invention since the ability of such polypeptides to confer any desired phenotypes on plants is neither disclosed nor suggested.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,

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